UNIVERSITI PUTRA MALAYSIA

MOLECULAR MARKERS AND THEIR APPLICATIONS IN THE CONSTRUCTION OF GENETIC LINKAGE MAPS AND ANALYSIS OF MONOGENIC AND QUANTITATIVE TRAITS IN OIL PALM

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By

RAJINDER SINGH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

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DEDICATION

To my parents, wife and children for their patience and love

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctorate of Philosophy

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By

Rajinder Singh

July 2005

Chairman: Professor Dr. Tan Soon Guan, PhD

Faculty: Faculty of Science

Molecular breeding is well suited to a perennial crop, like oil palm, in which the economic products are not produced until several years after planting. The use of DNA markers for selection in such crops could greatly reduce the number of breeding cycles needed. As such, the primary aim of this project was to map the oil palm genome by using restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and microsatellite (SSR) markers, in order to provide markers for selection in the breeding programme. A progeny derived from the selfing of a *tenera guineensis* palm (palm T128), which was segregating for the economically important monogenic characters of shell thickness and fruit color, was used for linkage map construction. A total of 523 segregating markers (117 RFLP, 22 SSR and 384 AFLP) were mapped to 17 linkage groups. In the current linkage map for the T128 palm, we have successfully mapped both the shell (*Sh*) gene and the fruit color (*Vir*) gene. Two RFLP markers mapped close to the *Vir* gene at a distance of 3 and 4 centiMorgans (cM), respectively. These two RFLP markers

were tested by using seven other independent crosses segregating for this trait. The markers could predict the trait with about 95% certainty. The *Sh* gene was located about 8 cM from the nearest marker (EAGG/MCTT-250, an AFLP marker). The AFLP marker could distinguish the *pisifera* palms (absence of shell) from the *dura* (thick shell) and *tenera* palms (thin shell) in the mapping family evaluated. These markers are useful tools for application in a molecular breeding programme for oil palm. Analysis was also extended to include mapping of quantitative traits (QTLs) associated with oil quality. Oil quality is determined by the fatty acid composition (FAC) in oil palm. By using the genome wide threshold levels calculated independently for each of the traits, significant QTLs were identified for myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1) and stearic acid (C18:0). In the attempt at mapping additional QTLs associated with oil quality, an interspecific cross between a Colombian *oleifera* (UP1026) and the T128 palm was also examined. A map consisting of 412 markers (302 AFLP, 83 RFLP and 27 SSR) in 18 linkage groups was generated at a LOD score of 5.0. At a genome wide significance threshold of $P<0.01$ and $P<0.05$, significant QTLs were detected for iodine value (IV), C14:0, C16:0, C16:1 and C18:0. There were common markers revealing significant linkages for the same trait in both the mapping families analyzed. Such tagging of markers to economically important traits will aid in expediting the production of elite planting materials with greater precision. To facilitate oil palm genome analysis leading to physical mapping and the identification of molecular markers associated with QTL and map based cloning, we attempted to develop the tools and techniques needed to construct a bacterial artificial chromosome (BAC) library for oil palm. A suitable method to purify and prepare the single copy vector (pBeloBAC11) for BAC transformation was

established. The proper partial digestion conditions of oil palm megabase DNA for BAC library construction were also determined. Several oil palm BAC clones were successfully identified. Hybridization of these BAC clones with oil palm DNA as probe confirmed the presence of oil palm DNA in those clones. In map based cloning efforts, cosmid libraries (with cloning capacity of up to 50 kb) are useful tools to complement BAC libraries. For this reason we constructed a cosmid library for oil palm. The library contains 65,000 clones with insert size ranging from 30 kb to 42 kb. Hybridization of randomly selected cosmid clones with oil palm DNA also confirmed the presence of oil palm DNA in these clones.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Falsafah Kedoktoran

PENANDA MOLEKUL DAN APLIKASINYA DALAM PEMBINAAN RANGKAIAN GENETIK SERTA ANALISIS MONOGENIK DAN CIRI KUANTITATIF DALAM KELAPA SAWIT

Oleh

Rajinder Singh

Julai 2005

Pengerusi: Profesor Dr. Tan Soon Guan,PhD

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Biakbaka molekul amat bersesuaian dengan tumbuhan saka seperti kelapa sawit di mana penghasilan produk ekonomi tidak akan terhasil sehingga beberapa tahun selepas penanaman. Penggunaan penanda DNA (asid deoksiribonukleik) untuk pemilihan dalam tanaman seperti ini, dapat mengurangkan bilangan kitaran biakbaka yang diperlukan. Oleh itu, tujuan utama projek ini adalah untuk memetakan genom kelapa sawit dengan menggunakan teknik-teknik seperti RFLP ("restriction fragment length polymorphism"), AFLP ("amplified fragment length polymorphism") dan penanda mikrosatelit (SSR) dalam usaha menyediakan penanda untuk pemilihan dalam program biakbaka. Kacukan sendiri satu pokok tenera *guineensis* (pokok T128) yang didapati memberikan perbezaan ciri-ciri monogenik yang amat penting daripada segi ekonomi seperti ketebalan tempurung dan warna buah, telah digunakan untuk pemetaan genetik. Sejumlah 523 petanda informatif (117 RFLP, 22 SSR dan 384 AFLP) berjaya dipetakan kepada 17 kumpulan rangkaian. Pada peta rangkaian T128 tersebut, gen-gen berkaitan ketebalan

tempurung (*Sh*) dan warna buah (*Vir*) telah berjaya dipetakan. Dua penanda RFLP dipetakan berdekatan dengan gen *Vir* pada jarak 3 dan 4 centiMorgans (cM) masing-masing. Kedua-dua penanda RFLP tersebut telah diuji dengan menggunakan tujuh kacukan lain yang juga memberikan perbezaan pada ciri tersebut. Penanda-penanda tersebut boleh meramalkan 95% kehadiran ciri tersebut. Manakala gen *Sh* didapati berada 8 cM dari penanda yang berhampiran (EAGG/MCTT-250, penanda AFLP). Penanda AFLP ini berkeupayaan membezakan pokok *pisifera* (ketidakhadiran tempurung) daripada pokok *dura* (tempurung tebal) dan pokok *tenera* (tempurung nipis) dalam famili pemetaan yang diuji. Penanda-penanda ini amat berguna dalam aplikasi program biakbaka molekul kelapa sawit. Analisis juga dilanjutkan untuk memetakan lokus ciri-ciri kuantitatif (QTLs) yang berkaitan dengan kualiti minyak. Kualiti minyak kelapa sawit ditentukan oleh komposisi asid lemak (FAC). Dengan menggunakan tahap signifikasi genome yang dikira secara berasingan untuk setiap ciri, QTLs bererti dikenalpasti untuk asid miristik (C14:0), asid palmitik (C16:0), asid palmitoleik (C16:1) dan asid stearik (C18:0). Dalam percubaan pemetaan QTLs tambahan berkaitan dengan kualiti minyak, satu kacukkan interspesifik di antara *oleifera* Colombia (UP1026) dan T128 juga telah dikaji. Satu peta yang mengandungi 412 penanda (302 AFLP, 83 RFLP dan 27 SSR) dalam 18 kumpulan rangkaian telah dijana pada skor LOD bersamaan 5.0. Pada ambang signifikan genome $P<0.01$ dan $P<0.05$, QTL yang signifikan telah dikesan untuk nilai iodin (IV), C14:0, C16:0, C16:1 dan C18:0. Terdapat penanda yang sama mendedahkan rangkaian signifikan untuk ciri yang sama dalam kedua-dua keluarga pemetaan yang dikaji. Penandaan penanda terhadap ciri kepentingan ekonomi dapat membantu mempercepatkan penghasilan bahan tanaman elit dengan lebih jitu. Untuk membantu analisis genom kelapa sawit ke arah pemetaan fizikal dan

pengenalpastian penanda molekul yang berkaitan dengan QTL dan pengklonan berasaskan peta, kami telah membangunkan peralatan dan teknik yang diperlukan untuk membina perpustakaan kromosom bakteria buatan (BAC) kelapa sawit. Satu kaedah yang sesuai untuk menulenkan dan menyediakan vektor salinan tunggal (pBeloBAC11) untuk transformasi BAC telah diasaskan. Suatu keadaan pencernaan separa teratur DNA megabes kelapa sawit untuk pembinaan perpustakaan BAC telah ditentukan. Beberapa klon BAC kelapa sawit berjaya dikenalpasti. Penghibridan klon-klon BAC ini dengan DNA kelapa sawit sebagai prob mengesahkan kehadiran DNA kelapa sawit di dalam klon-klon tersebut. Dalam usaha pengklonan berasaskan peta, perpustakaan-perpustakaan kosmid (dengan kapisiti pengklonan sehingga 50 kilobes) merupakan asas yang berguna kepada komplimentari perpustakaan BAC. Untuk tujuan ini, kami telah membina satu perpustakaan kosmid untuk kelapa sawit. Perpustakaan ini mengandungi 65, 000 klon dengan saiz selitan di antara 30 hingga 42 kb. Penghibridan beberapa klon kosmid terpilih dengan DNA kelapa sawit juga mengesahkan kehadiran DNA kelapa sawit di dalam klon-klon ini.

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I certify that an Examination Committee met on 6th July 2005 to conduct the final examination of Rajinder Singh on his Doctor of Philosophy thesis entitled "Molecular Markers and their Applications in the Construction of Genetic Linkage Maps and Analysis of Monogenic and Quantitative Traits in Oil Palm" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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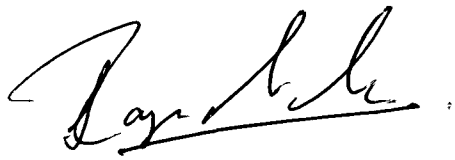
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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Date: 9/8/2005

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